

AMENDMENT UNDER 37 C.F.R. § 1.116
U.S. Appln. No. 09/786,627

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (Original): A method of measuring a concentration of an analyte in a sample, wherein said method comprises:

a) irradiating said sample with modulated incident light wherein said sample comprises a first fluorophore which absorbs a portion of said incident light and then emits emitted light, wherein the portion of said incident light absorbed by said first fluorophore is sensitive to the concentration of the analyte in the sample;

b) allowing said emitted light to irradiate a second fluorophore;

c) measuring light emitted from said sample;

d) determining the phase angle or modulation of said light emitted from said sample; and

e) correlating said phase angle or modulation to said concentration of said analyte,

wherein in said first fluorophore has a decay time on a nanosecond timescale,

wherein said second fluorophore has a decay time on a microsecond timescale.

2. (Original): The method of claim 1 wherein said modulated light is modulated at a frequency between 10 KHz and 100 MHz.

3. (Original) The method of claim 1 wherein said modulated light is modulated at a frequency between 50 KHz and 10 MHz.

4. (Original) The method of claim 1 wherein said modulated light is modulated at a frequency between 1 MHz and 10 MHz.

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5. (Original): The method of claim 1 wherein said first fluorophore is a naturally occurring component of said sample.

6. (Original): The method of claim 1 wherein said first fluorophore is added to said sample.

7. (Cancelled).

8. (Cancelled).

9. (Original): The method of claim 1 wherein said second fluorophore is a naturally occurring component in said sample.

10. (Original): The method of claim 1 wherein said second fluorophore is added to said sample.

11. (Original): The method of claim 1 wherein said second fluorophore is separate from said sample.

12. (Original): The method of claim 1 wherein a probe for measuring said light emitted from said sample comprises said second fluorophore.

13. (Original): The method of claim 1 wherein said second fluorophore is on a container containing said sample.

14. (Original): The method of claim 1 wherein said analyte is in vivo, blood plasma, whole blood, saliva or body fluid.

15. (Original): The method of claim 14 wherein said second fluorophore is placed onto the outside of an organism comprising said analyte.

16. (Original): The method of claim 1 wherein said analyte is selected from the group consisting of H^+ , pH, Na^+ , K^+ , Li^+ , Mg^{2+} , Ca^{2+} , Cl^- , HCO_3^- , CO_2 , O_2 , glucose, lactate, an antigen

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and a drug.

17. (Previously Presented): The method of claim 1 wherein said first fluorophore is selected from the group consisting of Quin-2 (Glycine,N-(2-((8-(bis(carboxymethyl)amino)-6-methoxy-2-quinoliny)methoxy)-4-methylphenyl)-N-(carboxymethyl)-), Fura-2 (5-Oxazolecarboxylic acid, 2-(6-(bis(carboxymethyl)amino)-5-(2-(2-(bis(carboxymethyl)amino)-5-methylphenoxy)ethoxy)-2-benzofuranyl)-, pentapotassium salt], Indo-1 (1H-Indole-6-carboxylic acid, 2-[4-bis-(carboxymethyl)amino]-3-[2-[2- (bis-carboxymethyl)amino-5-methylphenoxy]ethoxy]phenyl]-, pentapotassium salt), Calcium Green (Glycine, N-[2-[2-[2-[bis(carboxymethyl)amino]-5- [[2',7'-dichloro-3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H), 9'- [9H]xanthene)-5- yl]carbonyl]amino]phenoxy]ethoxy]phenyl]-N- (carboxymethyl)-, hexapotassium salt), Calcium Orange (Xanthylium, 9-[4-[[[4-[bis[2- [(acetyloxy)methoxy]- 2-oxoethyl]amino]-3-[2-[2-[bis[2- [(acetyloxy)methoxy]-2-oxoethyl]amino]phenoxy]ethoxy]phenyl]amino]thioxomethyl]amino]- 2-carboxyphenyl]- 3,6-bis(dimethylamino)-, inner salt), Calcium Crimson, Benzoxazine-crown, Mag-Quin-2 , Magnesium Green (Glycine, N-[2-(carboxymethoxy)-4-[[2',7'-dichloro-3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthene]-5-yl]carbonyl]amino]phenyl]-N- (carboxymethyl)-, pentapotassium salt), [[Benzoxazine-crown,]] PBFI (1,3-Benzenedicarboxylic acid, 4,4'-[1,4,10,13-tetraoxa-7,16- diazacyclooctadecane-7,16-diylbis(5-methoxy-6,2-benzofurandiyl)]bis-), Sodium Green (Spiro[isobenzofuran-1(3H),9'- [9H]xanthene]-5-carboxamide, N,N⁺-[1,4,10-trioxa-7,13- diazacyclopentadecane-7,13-diylbis(2,5-dimethoxy-4,1-phenylene)]bis[3',6'- bis(acetyloxy)-2',7'-dichloro-3-oxo), SNAFL-1 (Spiro[7H-benzo[c]xanthene-7,1'(3'H)-isobenzofuran]-ar'-carboxylic acid, 3,10-dihydroxy-3'oxo-), C.

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SNAFL-1, C. SNAFL-2, C. SNARF-1 (Benzenedicarboxylic acid, 2(or 4)-[10-(dimethylamino)-3-oxo-3H- benzo[c]xanthene-7-yl]-), C. SNARF-2, C. SNARF-6, C. SNARF-X, BCECF (2',7'-bis-(2-carboxyethyl)-5-(and-6-)- carboxylfluorescein) Spiro(isobenzofuran-1(3H), 9'-(9H) xanthene)-2',7'-dipropanoic acid, ar-carboxy-3',6'-dihydroxy-3-oxo-), Resorufin Acetate (3H-Phenoxazin-3-one, 7-acetate-), 6-methoxy-*N*-ethylquinolinium chloride, *N*-(6-methoxyquinolyl)acetoethyl ester, 6-methoxy-*N*-ethylquinolinium chloride, 6-methoxy-*N*-(3-trimethylammoniumpropyl)quinolinium dibromide, 6-methoxy-*N*-(3-trimethylanunoniumpropyl)phenanthrindium dibromide, 6-methoxy-*N*-(4-arninoalkyl)quinolinium, bromide hydrochloride, 6-methoxy-*N*-(3-sulfoproxyl)quinolinium *N*-sulfopropylacridinium, *N,N*'-dimethyl-9,9'-bisacridinium nitrate, *N*-methylacridinium-9-carboxamides, *N*-methylacridinium-9-methylcarboxylate, 8-hydroxypyrene-1,3,6-trisulfanate, [Ru(4,4'-diethylamin` omethyl-2,2'-bipyridine)(2,2'-bipyridine)₂]²⁺, Oregon Green Spiro[isobenzofuran-1(3H),9'- [9H]xanthen]-3-one, 2',7'-difluro-3',6'-dihydroxy-), DM-NERF Spiro(isobenzofuran-1(3H),9'-(9H) xanthene)-ar- carboxylic acid, 2',7'-dimethyl-3'-ethylamino-6'-hydroxy-3-oxo-), Cl-NERF (Spiro(isobenzofuran-1(3H),9'-(9H) xanthene)-ar-carboxylic acid, 2'-chloro -6'-ethylamino-3'-hydroxy-7'-methyl-3-oxo-), Mag-Quin-1, Mag-Fura-2 (5-Oxazolecarboxylic acid, 2-[6-[bis(carboxymethyl)amino]-5- (carboxymethoxy)-2-benzofuranyl]-, tetrapotassium salt), Mag-Fura-5, Mag-Indo-1 (1H-Indole-6-carboxylic acid, 2-[4-[bis(carboxymethyl)amino]-3- (carboxymethoxy)phenyl]-, tetrapotassium salt), Mag-Fura-Red, Mg Orange (Xanthylium, 9-[4-[[[[3-carboxymethoxy-4-(carboxymethyl)amino]phenyl]amino]thioxomethyl]amino]-2-carboxyphenyl]-3,6-bis(dimethylamino)-, tripotassium salt), sodium-binding benzofuran isophthalate, sodium-

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binding benzofuran oxazole, CD222, Fura Red (Glycine, N-[2-[(acetyloxy)methoxy]-2-oxoethyl]-N-[5-[2-[2-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-5-methylphenoxy]ethoxy]-2-[(5-oxo-2-thioxo-4-imidazolidinylidene)methyl]-6-benzofuranyl]-, (acetyloxy)methyl ester), BTC (coumarin benzothiazole-based indicator) (Glycine, N-[3-(2-benzothiazolyl)-6-[2[2-[bis(carboxymethyl)amino]-5-methylphenoxy]ethoxy]-2-oxo-2H-1-benzopyran-7-yl]-N-(carboxymethyl)-, tetrapotassium salt), Fluo-3 (Glycine, N-[2-[[[2-[bis(carboxymethyl)amino]-5-(2,7-dichloro-6-hydroxy-3-oxo-3H-xanthen-9-yl)phenoxy]methyl]oxy]-4-methylphenyl]-N-(carboxymethyl)-, pentammonium salt), Rhod-2 (Xanthylum, 9-[4-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-3-[2-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]phenoxy]ethoxy]phenyl]-3,6-bis(dimethylamino)-, bromide), Ca Green-2 (Glycine, N,N'-[1,2-ethanediylbis[oxy[4-[(2',7'-dichloro-3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)carbonyl]amino]-2,1-phenylene]]]bis[N-(carboxymethyl)-, octapotassium salt), Ca Green-5N (Glycine, N-[2-[2-[2-[bis(carboxymethyl)amino]-5-[(2',7'-dichloro-3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)carbonyl]amino]phenoxy]ethoxy]-4-nitrophenyl]-N-(carboxymethyl)-, hexapotassium salt) Ca Orange-5N (Xanthylum, 9-[4-[[[4-[bis(carboxymethyl)amino]-3-[2-[2-[bis(carboxymethyl)amino]4-nitrophenoxy]ethoxy]phenyl]amino]thioxomethyl]amino]-2-carboxylphenyl]-3,6-bis(dimethylamino)-, tetrapotassium salt), Oregon Green - BAPTA-1 (Glycine, N-[2-[(acetyloxy)methoxy]-2-oxoethyl]-N-[4-[[[3',6'-bis(acetyloxy)-2',7'-difluoro-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl]carbonyl]amino]-2-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]phenoxy]ethoxy]phenyl]-, (acetyloxy)methyl ester),

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BAPTA-2 1,2-bis[3',6'-bis(acetyloxy)-2',7'-difluoro-3-oxospiro[isobenzofuran-1(3H), 9'-[9H]xanthen]-5-yl]carbonyl]amino]-2- [2-[2-[bis[2- [(acetyloxy)methoxy]-2-oxoethyl]amino]phenoxy-ethan) and BAPTA-5N (Glycine, N-[2-[2-[2-[bis(carboxymethyl)amino]-5- [[(2',7'-difluoro-3',6'-dihydroxy-3- oxospiro[isobenzofuran-1(3H),9'- [9H]xanthen]-5-yl) carbonyl]amino]phenoxy[ethoxy]-4- nitrophenyl]-N-(carboxymethyl)-, hexapotassium salt).

18. (Previously Presented): The method of claim 1 wherein said second fluorophore is a compound selected from at least one of:

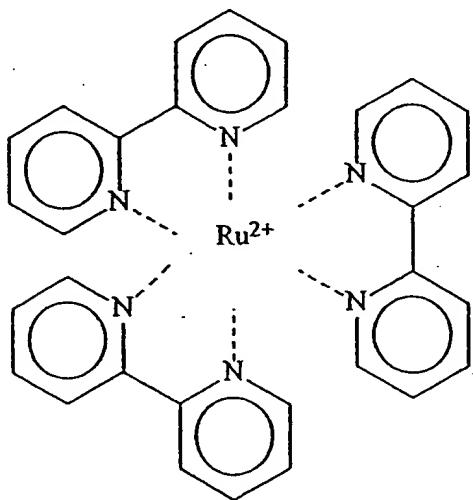


Figure 20A

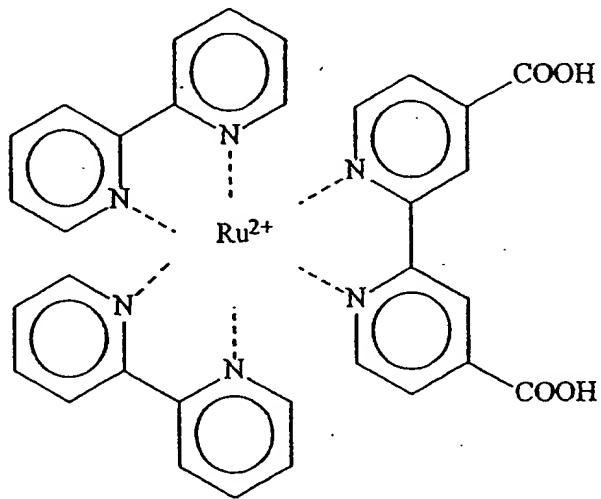


Figure 20B

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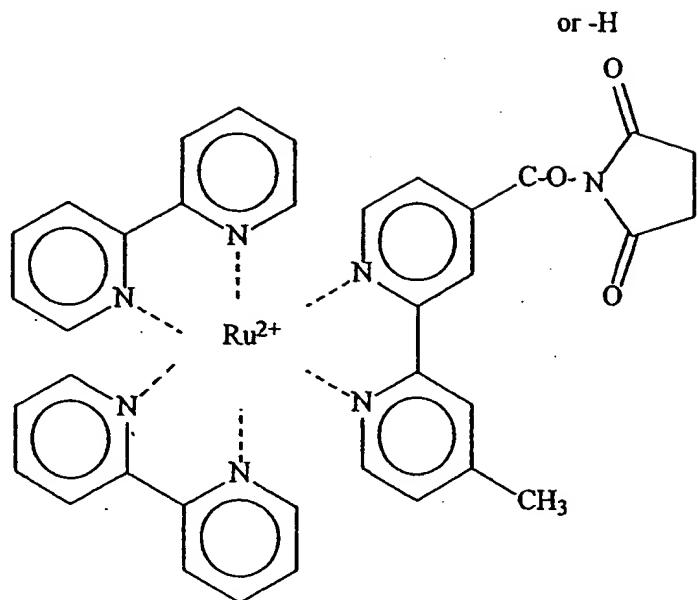


Figure 20C

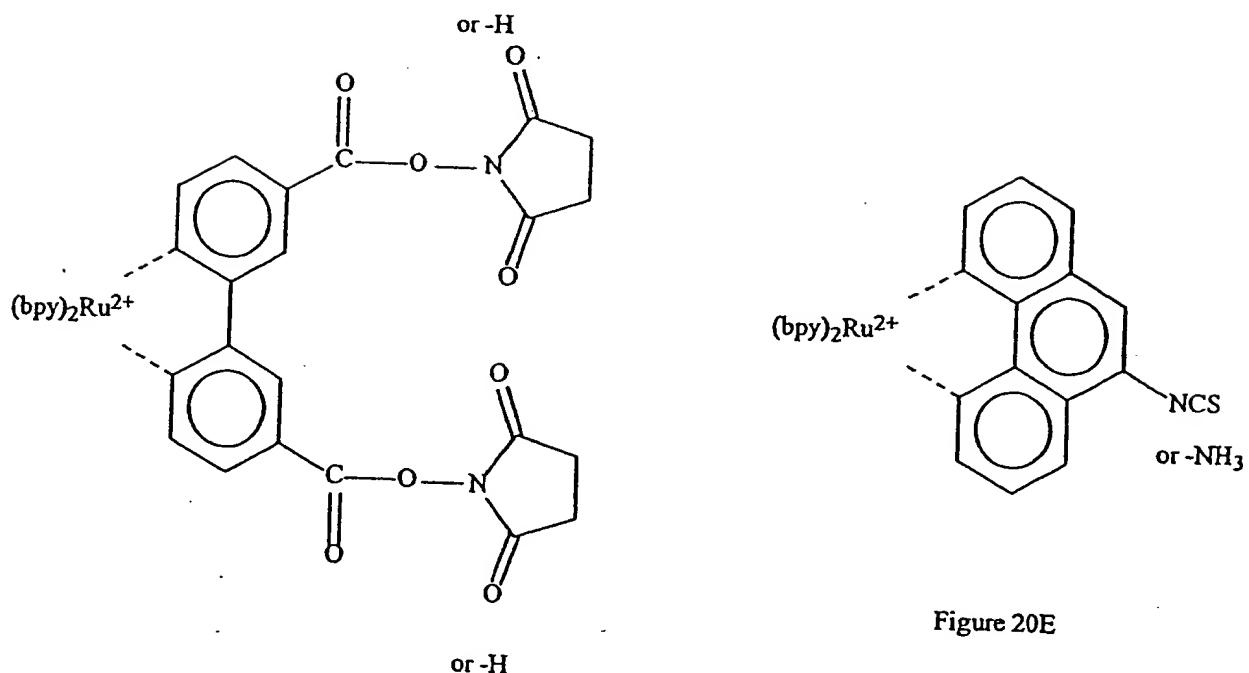


Figure 20E

Figure 20D

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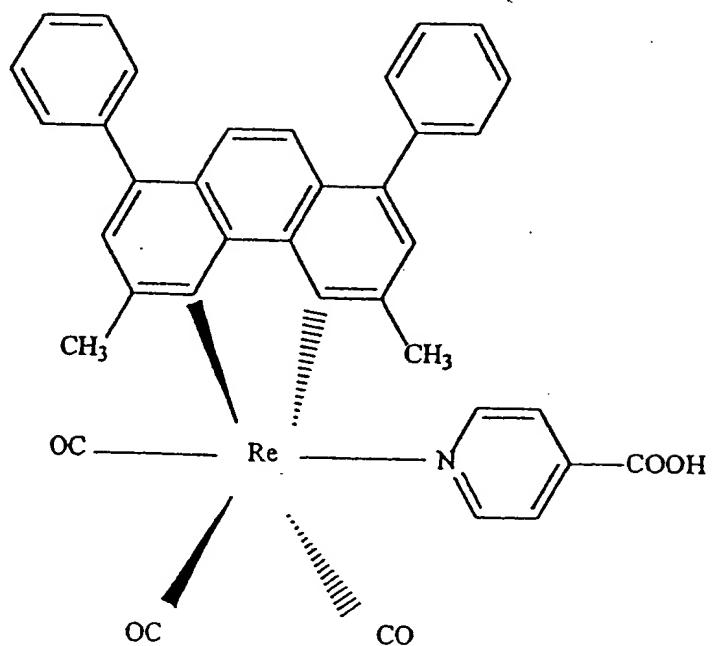


Figure 20F

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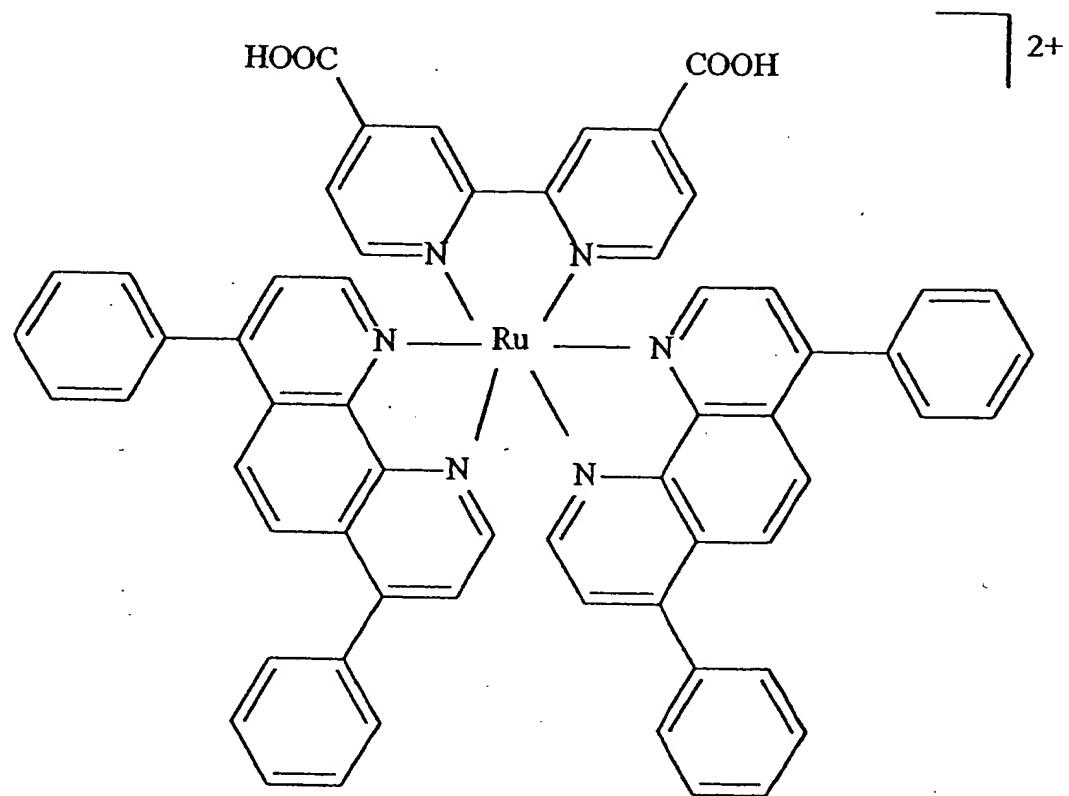


Figure 20G

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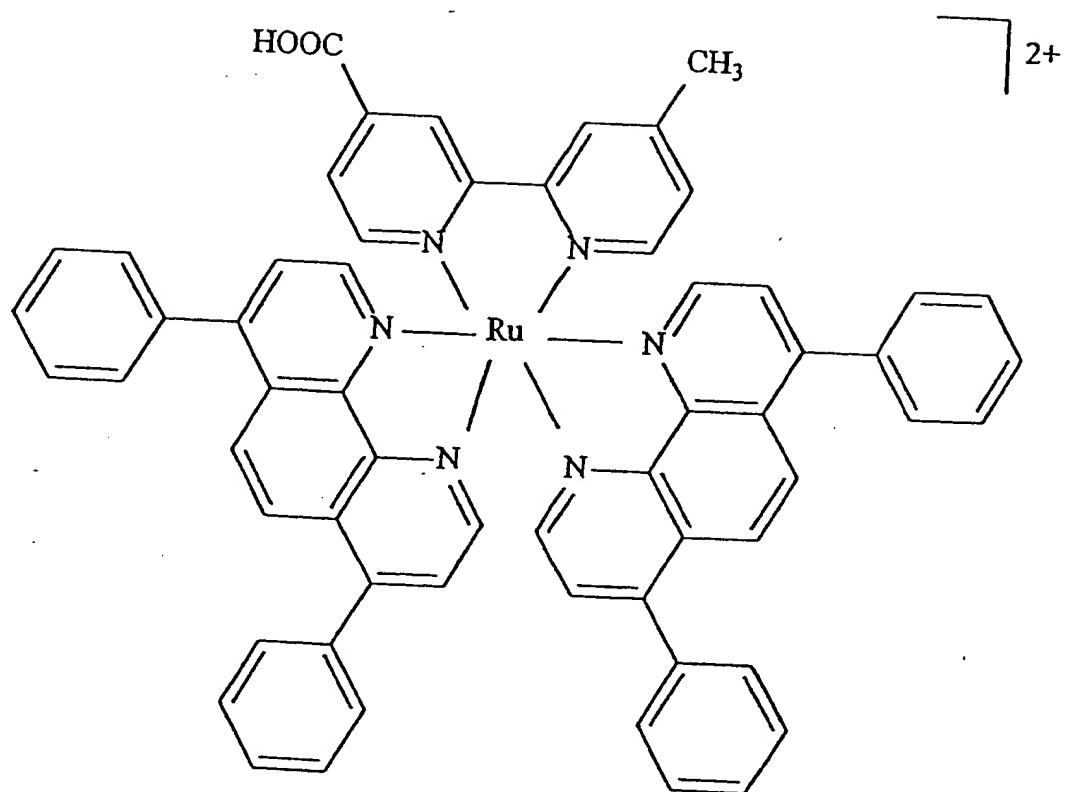


Figure 20H

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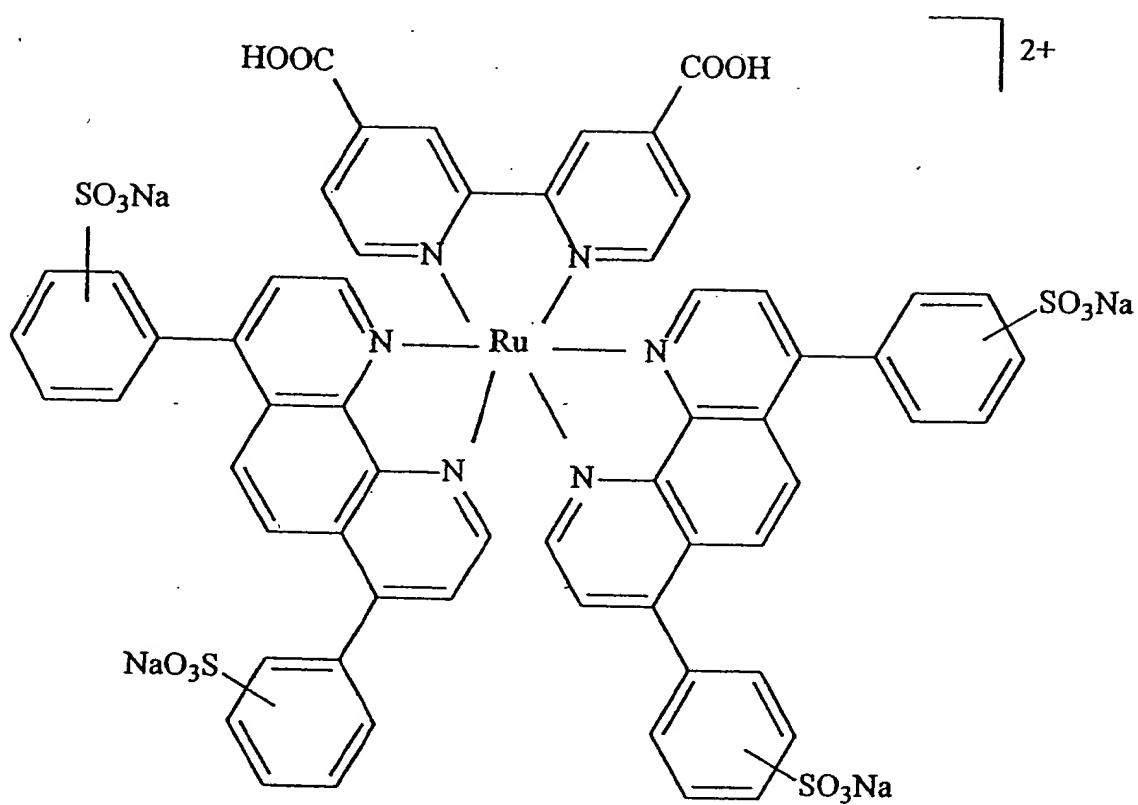


Figure 20I

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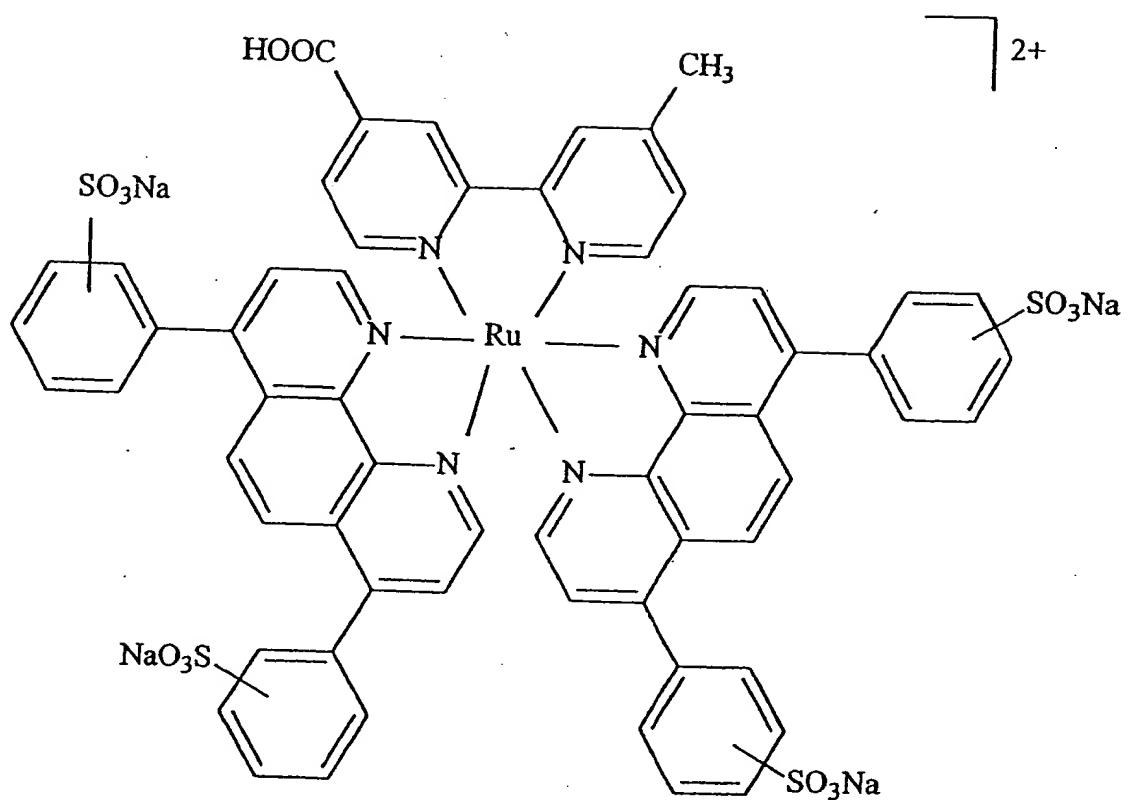


Figure 20J

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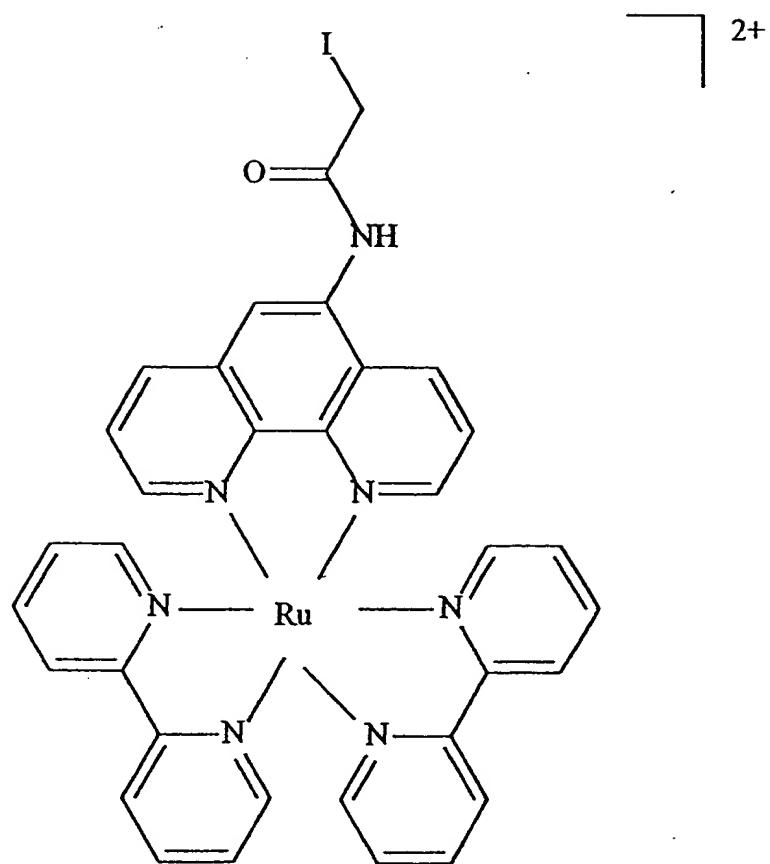


Figure 20K

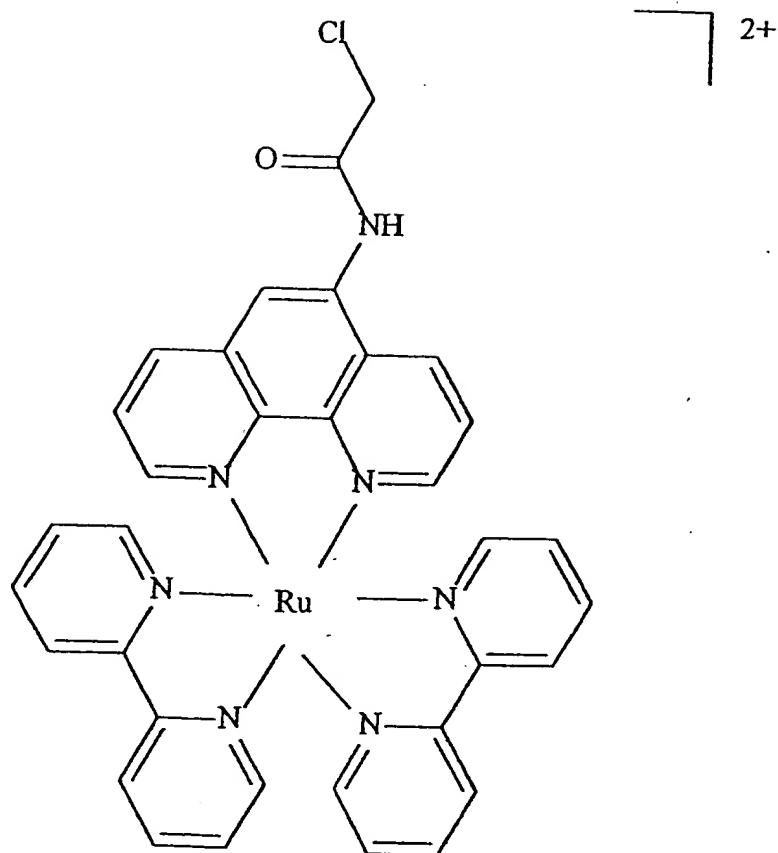


Figure 20L

19. (Original): The method of claim 1 further comprising inserting a filter between said light emitted from said sample and a detector wherein the percentage of light emitted from said first fluorophore which is absorbed by said filter is different than the percentage of light emitted from said second fluorophore which is absorbed by said filter.

20. (Original): The method of claim 19 wherein the percentage of light emitted from said first fluorophore which is absorbed by said filter is greater than the percentage of light emitted from said second fluorophore which is absorbed by said filter.

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21. (Original): The method of claim 19 wherein multiple measurements are made wherein measurements are made i) with different filters or ii) with one or more filters and with no filter.
22. (Original): The method of claim 1 wherein measuring is performed at more than one frequency.
23. (Original): The method of claim 1 wherein said sample comprises a light scattering medium.
24. (Original): The method of claim 23 wherein said light scattering medium is skin.
25. (Original): The method of claim 1 wherein said method is used clinically.
26. (Original): The method of claim 16, wherein said analyte is glucose, further wherein said second fluorophore is a glucose-sensitive fluorophore or a glucose-binding protein.
27. (Original): The method of claim 26 wherein said glucose-binding protein is a glucose-galactose binding protein or concanavalin A.
28. (Original): The method of claim 27 wherein said glucose-galactose binding protein or concanavalin A is labeled with a fluorophore.
29. (Original): The method of claim 16 wherein said analyte is an antigen or a drug, further wherein said second fluorophore is an antibody labeled with a fluorescent compound or said second fluorophore is an antibody fragment labeled with a fluorescent compound.
30. (Original): The method of claim 16 wherein said analyte is lactate, further wherein said second fluorophore is a lactate-specific fluorophore or a lactate binding protein labeled with a fluorescent compound.
31. (Original): The method of claim 1 wherein said incident light is produced by a laser,

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a light emitting diode (LED) or an electroluminescent light source (ELL).

32. (Original): The method of claim 1 wherein said sample is from a tissue culture or an aquarium.

33. (Original): The method of claim 1 wherein said method is used to monitor a bioprocessing reaction.

34. (Original): The method of claim 1 wherein said method is used as a part of an analytical chemistry process.

35. (Original): The method of claim 1 wherein said method is used industrially or in process control

36. (Original): The method of claim 1 wherein said first fluorophore is said analyte.

37 (Cancelled).

38. (Currently Amended): The method of claim [[37]] 1, wherein the detected emission of said first and second fluorophores is equivalent to a fraction of a total emission of the first fluorophore.

39. (Previously Presented): The method of claim 38, wherein said light is modulated at a frequency between 50 kHz and 10 MHz.

40. (Currently Amended): The method of claim [[37]] 1, wherein the first fluorophore exhibits no lifetime change in presence of the analyte.

41. (Previously Presented): The method of claim 39, wherein measuring is performed at more than one frequency.

42. (Cancelled).